Supplementary Information

9 Supplementary figures with legends

UCHL5 controls ß-catenin destruction complex function through Axin1 regulation

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# Contributed equally

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Figure S1

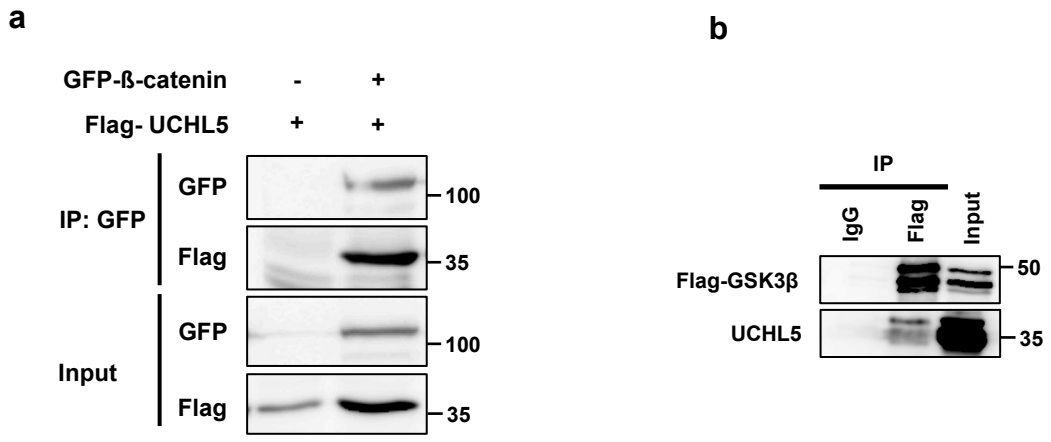

a
GFP-ß-catenin - +
Flag- UCHL5 + +
IP: GFP
GFP
Flag
100
35
Input
GFP
Flag
100
35
b
IP
IgG
Flag
Input
Flag-GSK3β
UCHL5
50
35

Figure S2

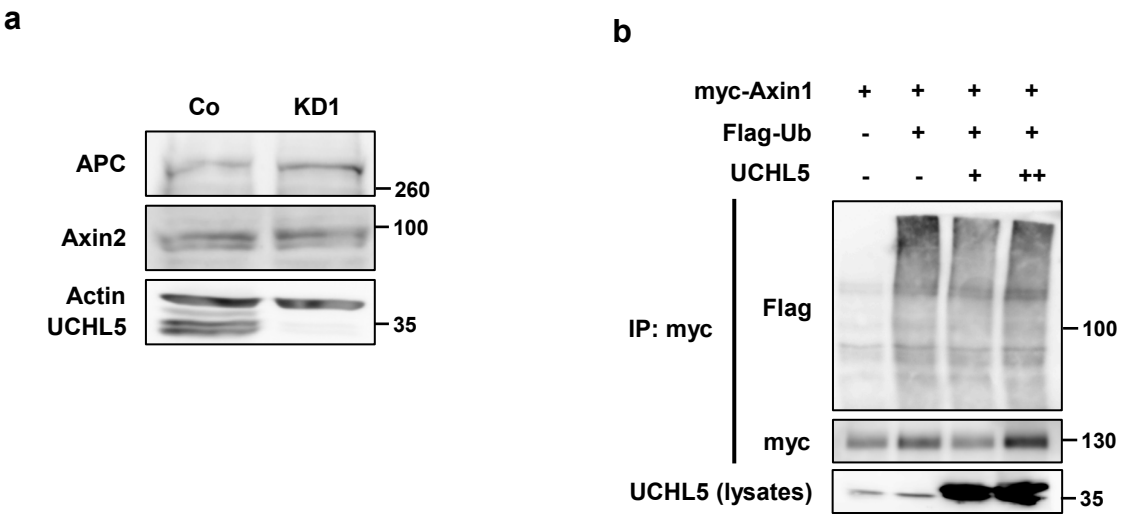

a
Co
KD1
APC
Axin2
Actin
UCHL5
260
100
35
b
myc-Axin1 + + + +
Flag-Ub - + + +
UCHL5 - - + ++
IP: myc
Flag
myc
UCHL5 (lysates)
100
130
35

Figure S3

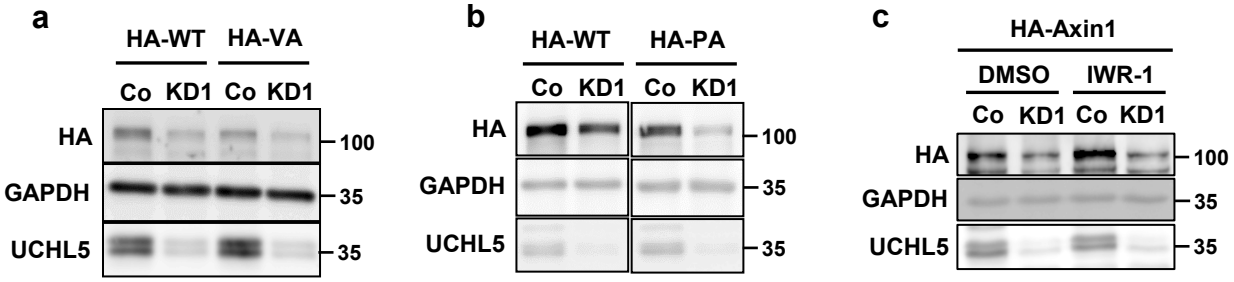

a
HA-WT
HA-VA
Co
KD1
Co
KD1
HA
GAPDH
UCHL5
100
35
35
b
HA-WT
HA-PA
Co
KD1
Co
KD1
HA
GAPDH
UCHL5
100
35
35
c
HA-Axin1
DMSO
IWR-1
Co
KD1
Co
KD1
HA
GAPDH
UCHL5
100
35
35

Figure S4

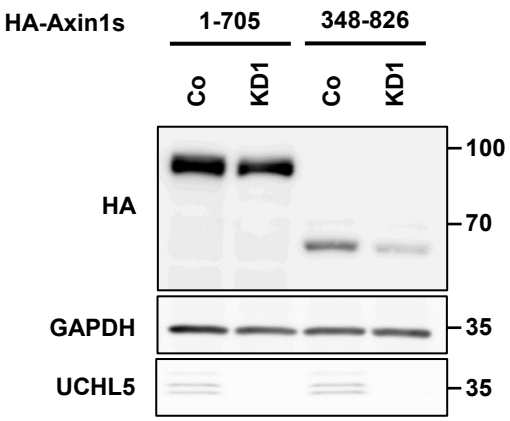


Figure S5

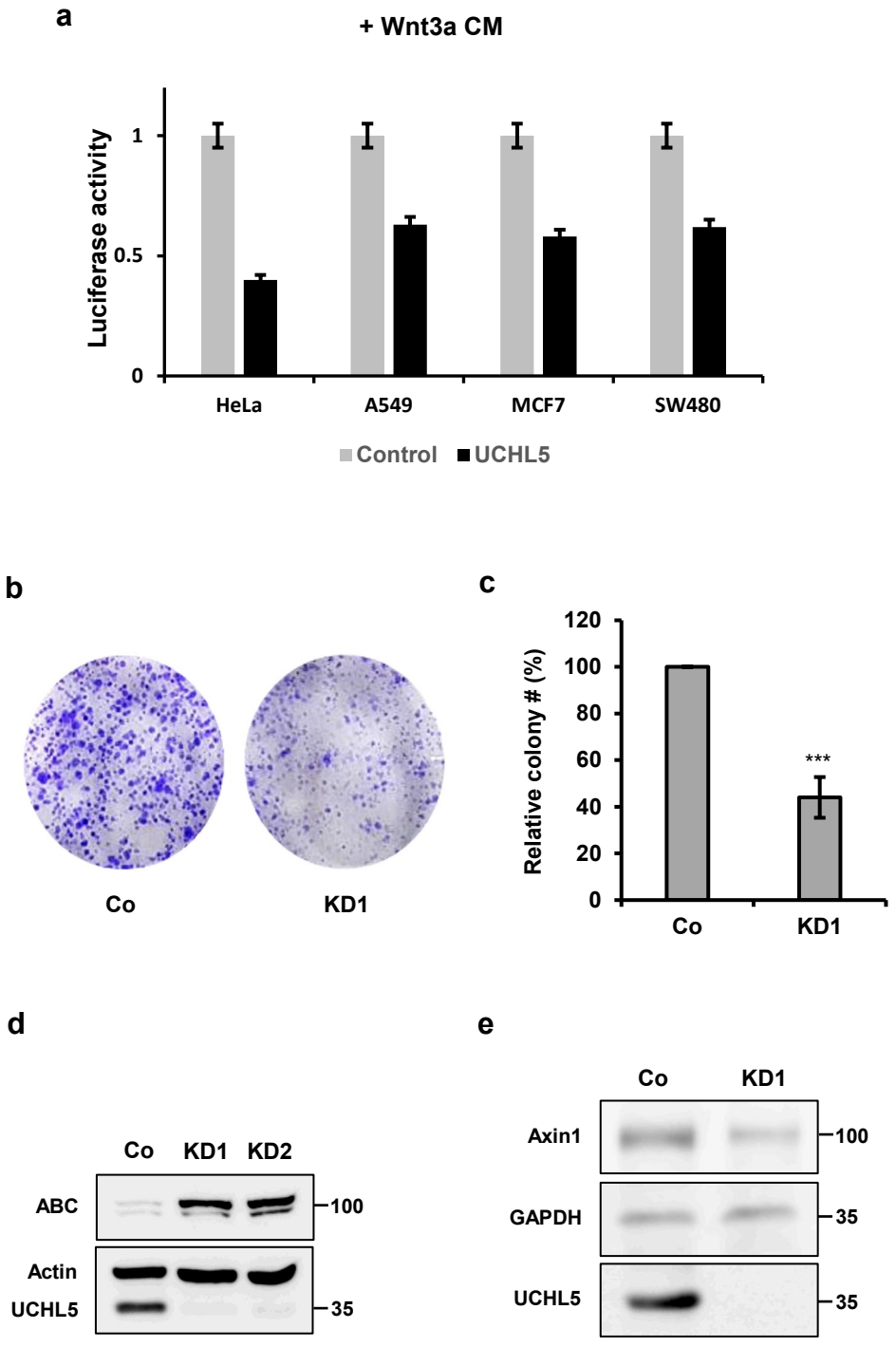

a
+ Wnt3a CM
Luciferase activity
1
0.5
0
HeLa
A549
MCF7
SW480
Control
UCHL5
b
Co
KD1
c
Relative colony # (%)
120
100
80
60
40
20
0
***
Co
KD1
d
Co
KD1
KD2
ABC
100
Actin
UCHL5
35
e
Co
KD1
Axin1
100
GAPDH
35
UCHL5
35

Figure S6

Figure 1c

Figure 1g

Figure 2b

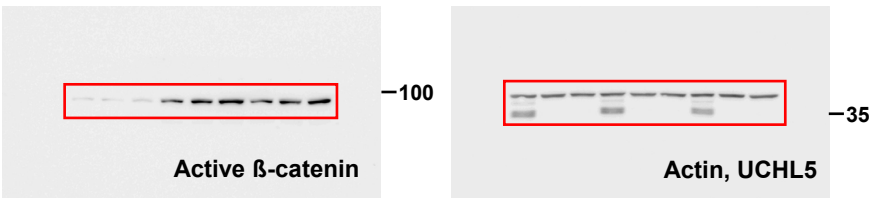


Figure 2c

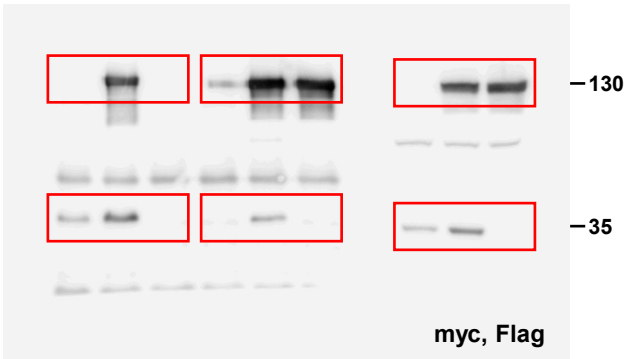


Figure 2d

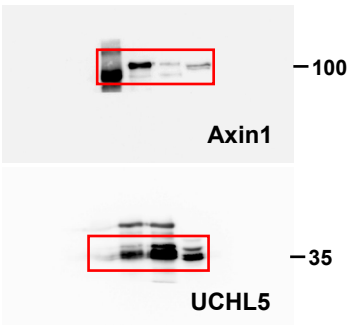


Figure 2f

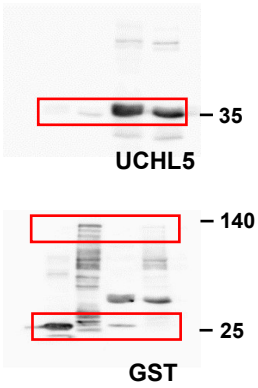


Figure 2h

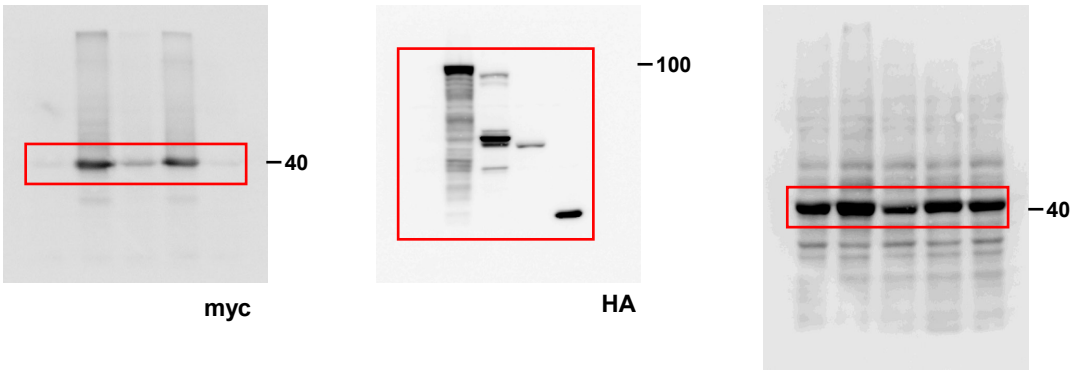


Figure S7

Figure 2i

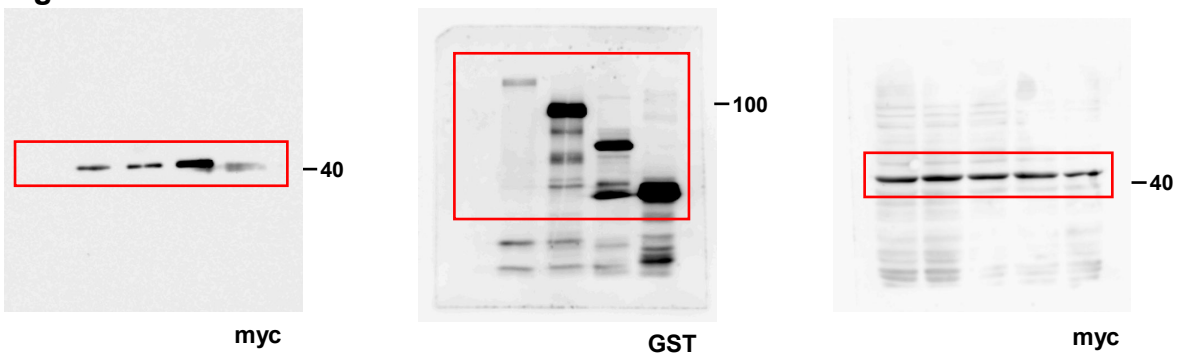


Figure 3a

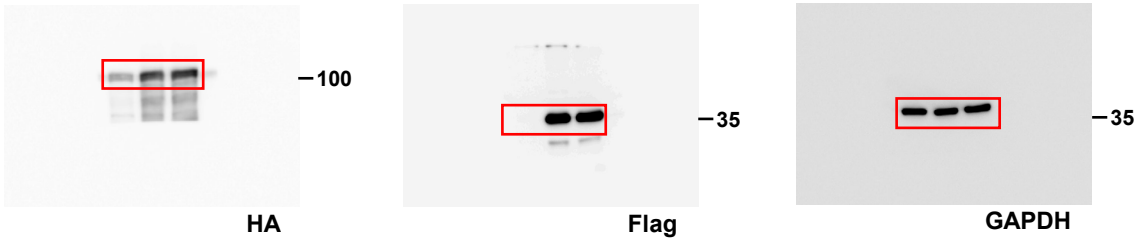


Figure 3b

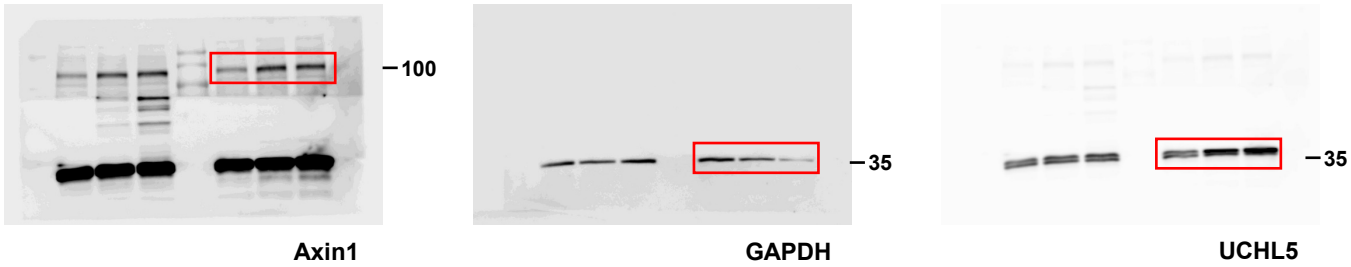


Figure 3c

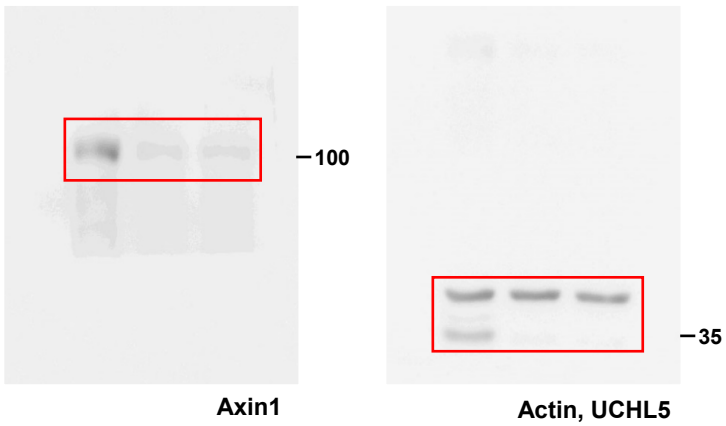
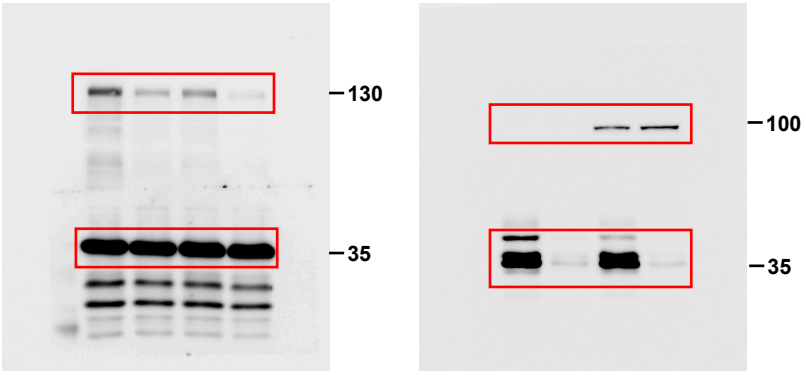


Figure 3d

Figure S8

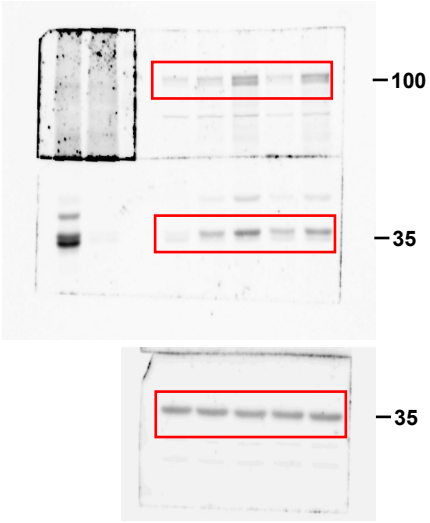
Figure 3f



myc, GAPDH

Active ß-catenin, UCHL5

Figure 3g



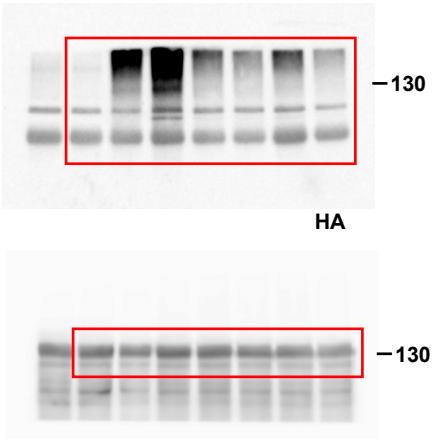
HA, UCHL5, GAPDH

Figure 3i

Axin1, Actin

UCHL5

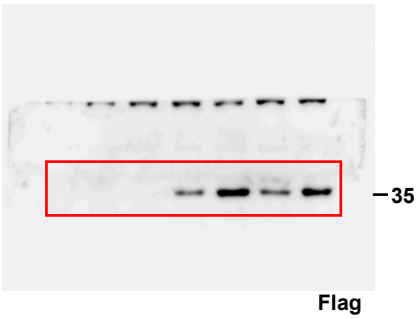
Figure 3j



HA

myc

myc



Flag

Figure S9

Figure 3k

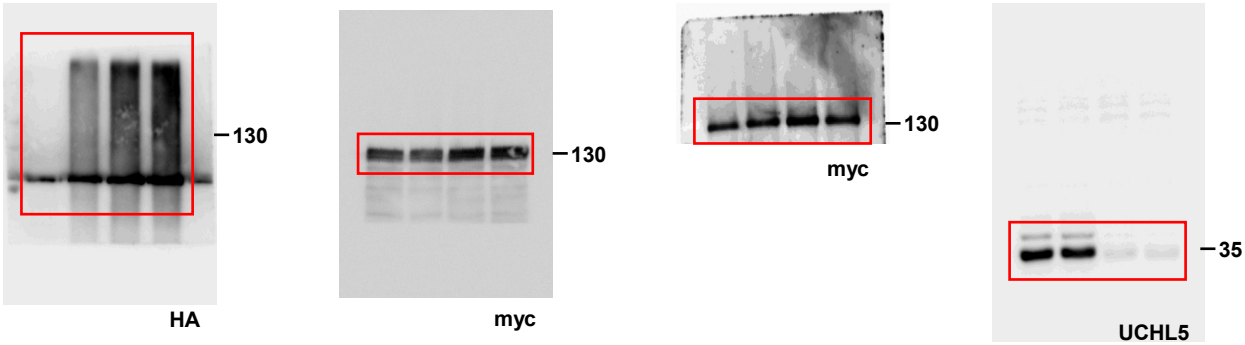


Figure 4b

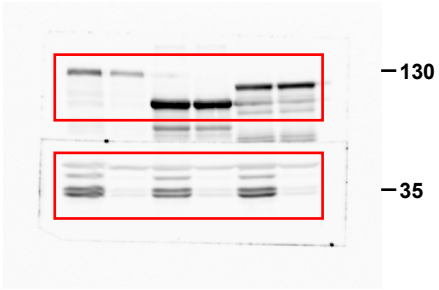


Figure 4c

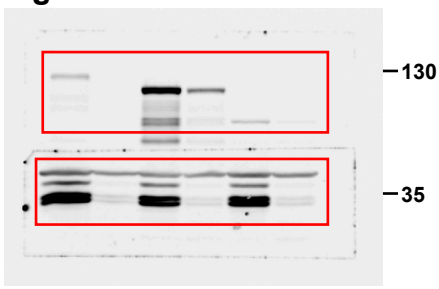


Figure 4e

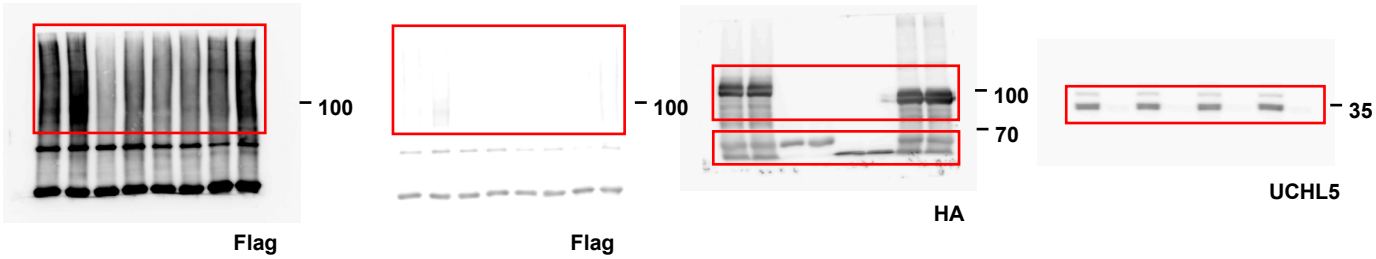


Figure 5c

Figure S1. UCHL5 interacts with components of ß-catenin destruction complex. a. IP assay using HeLa cells. Cells were transfected with Flag-UCHL5 (2μg) and GFP-ß-catenin (2μg). Then, cell lysates were subjected to immunoprecipitation with anti-GFP. **b.** IP assay using HeLa cells. Cells were transfected with Flag-GSK3ß (2μg). Then, cell lysates were subjected to immunoprecipitation with anti-Flag.

Figure S2. UCHL5 stabilizes Axin1, but its deubiquitinating activity is dispensable. a. Western blot analysis using Co and KD1 HeLa cells. Cell lysates were immunoblotted with endogenous APC, Axin2, Actin, and UCHL5 antibodies. **b.** *In vitro* ubiquitination assay. HeLa cells were co-transfected with both myc-Axin1 and Flag-Ub plasmids to express polyubiquitinated Axin1. After 48 h, cell lysates were immunoprecipitated using anti-myc antibody to obtain polyubiquitinated Axin1. Precipitated polyubiqutinated Axin1 was then incubated with UCHL5 recombinant proteins (+ 1ug; ++ 2ug).

Figure S3. UCHL5 stabilizes Axin1 in an independent mechanism from SIAH1/2 and Tankyrase. a, b. Western blot analysis using Co and KD1 HeLa cells. Cells were transfected with 0.5μg of Wild type Axin1 and two Axin1VxP mutants (HA-WT, HA-VA, and HA-PA). Cell lysates were then immunoblotted to detect the level of HA-Axin1, UCHL5, and GAPDH proteins. **c.** Western blot analysis using Co and KD1 HeLa cells. Cells were transfected with 0.5μg of HA-Axin1, then treated with either DMSO or IWR-1(12Mm) for 16hrs. Cell lysates were immunoblotted to detect the level of HA-Axin1, UCHL5, and GAPDH proteins.

Figure S4. UCHL5 mediates Axin1 stabilization through the DIX domain. 1μg of truncated Axin1 mutants (1-705 a.a., 348-826 a.a.) were transfected into WT and KD1 HeLa cells. Resulting cells lysates were subjected to immunoblotting with antibodies against HA, GAPDH, and UCHL5.

Figure S5. UCHL5 negatively regulates Wnt signal activity in various cancer cells. a. TOPflash assay using HeLa, A549, MCF7, and SW480. Cells were transfected with the indicated plasmids (50ng *TK-Renilla* reporter; 250ng TOPFlash; 250ng FOPFlash; 2μg pCS2+UCHL5). Then, Wnt3a CM was treated for 16hrs. **b, c.** Clonogenic assay and qualitative analysis of SW480 cells . 200 cells were seeded into a 6-well plate and cultured for 2 weeks until each cell formed colony. colonies were then fixed and stained with crystal violet (*** $p < 0.0005$). The data are presented as the mean ± SD of the experiments performed in triplicate. . **d, e.** Western blot analysis using Co and KD SW480 cells. Cell lysates were immunoblotted to detect the level of active β-catenin, Axin1, UCHL5, Actin, and GAPDH proteins

Figure S6-S9. Full-length images of gels and blots from the main figures.